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Overview of genomic and bioinformatic resources for *Zymoseptoria tritici*

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ABSTRACT

Zymoseptoria tritici (syn. *Mycosphaerella graminicola*, *Septoria tritici*) is a haploid fungus belonging to the class Dothideomycetes. It is the causal agent of septoria leaf blotch – one of the world's most significant diseases of wheat. Here we review the genomic and bioinformatic resources that have been generated for *Z. tritici*. These include the whole-genome reference assembly for isolate IPO323, genome resequencing of alternate isolates, mitochondrial genome sequences, transcriptome sequences and expression data, and annotations of gene structure and function. We also highlight important advances in our fundamental knowledge of genome evolution and its effects on adaptation and pathogenicity in *Z. tritici* that have been facilitated by these resources.

1. The nuclear genome

An early landmark paper studied the electrophoretic karyotype of 7 isolates of *Z. tritici*, which estimated 14–16 chromosomes ranging from 330 kb to 3.5 Mb (McDonald and Martinez, 1991). Chromosome length and number polymorphisms were also observed between isolates, with chromosome presence/absence variation (PAV) observed exclusively for the two shortest chromosome bands. Subsequent generation of a genetic map from a cross between parent isolates IPO323 and IPO94269 predicted a higher number of chromosomes at 23 linkage groups (Kema et al., 2002). Further analysis of this mapping population revealed that at least 8 of the smaller linkage groups – corresponding to 8 dispensable chromosomes ranging from 0.39 to 0.77 Mb – were not required for saprophytic growth i.e. were dispensable or accessory chromosomes (ACs) (Wittenberg et al., 2009). The distinction between core chromosomes (CCs) and ACs was an important milestone for *Z. tritici* genomics.

Z. tritici was the first species of the Dothideomycetes – a fungal class of high agricultural significance (Hane et al., 2011b; Ohm et al., 2012) – to have had the genome of a representative isolate (IPO323) (Kema and van Silfhout, 1997) sequenced beyond the typical 'draft genome' status, achieving near complete chromosome sequences spanning from telomere to telomere (Goodwin et al., 2011) (Table 1). The IPO323 reference genome assembly is 39.69 Mb in length, representing 21 chromosomes. At least half of the genome is contained within the six largest assembled sequences (that is, an N50 of 6), with the sixth largest sequence having a length of 2.67 Mb. Its chromosomes have been designated into CCs and ACs, numbered 1–13 and 14–21 respectively.

In addition to chromosomal polymorphisms across the ACs, the *Z. tritici* genome has also been observed to exhibit at least two other forms of genomic plasticity. The repetitive content of the IPO323 genome reference is 12.26% (Ohm et al., 2012) and its repeats exhibit the hallmarks of repeat-induced point mutation (RIP) (Goodwin et al., 2011) – a fungal specific type of mutation that targets repetitive DNA and randomly converts cytosine to thymine bases (Hane and Oliver, 2008, 2010; Hane et al., 2015). In comparisons of whole-genome sequences across species belonging to the sub-phylum Pezizomycotina, the *Z. tritici* genome also exhibits a typical "mesosyntenic" conservation pattern, that is, the relative order and orientation of genes are reshuffled within homologous chromosomes due to frequent intra-chromosomal recombinations (Croll et al., 2013; Hane et al., 2011a). The combined effects of AC variability, mesosynteny and RIP may have significant implications for genome evolution and pathogenicity in *Z. tritici*.

Due to the completeness of its genome assembly, *Z. tritici* IPO323 has been a key point of reference in several comparative genomics studies, particularly across the Dothideomycetes (Hane et al., 2011b; Morais do Amaral et al., 2012; Ohm et al., 2012). Observations of mesosynteny made from genome sequence

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alignments of different fungal species with *Z. tritici* can also be applied to fungal genome finishing. Whole-genome alignments of draft assemblies of other Pezizomycotina species with the *Z. tritici* IPO323 reference assembly have previously been used to predict the co-location of scaffolds on the same chromosome (i.e. synteny) (Goodwin et al., 2011). Significant insight has also been gleaned within the species *Z. tritici* itself through comparisons of alternate isolates, revealing differential selection pressures between CCs and ACs (Stukenbrock et al., 2010) and highlighting isolate-specific patterns of chromosome presence and absence (Goodwin et al., 2011; Croll et al., 2013) (see also McDonald et al., 1991).

2. The mitochondrial genome

The mitochondrial genomes (mtDNA) of two *Z. tritici* isolates, the reference isolate IPO323 and strain STBB1 (Table 1), have been sequenced in full (Torriani et al., 2008). The *Z. tritici* mtDNA [Nucleotide: EU090238] is 43,960 bp in length, indicating that it has been relatively untouched by invading intronic endonucleases (as compared with *Leptosphaeria maculans* and *Pyrenophora tritici-repentis*) (Manning et al., 2013; Rouxel et al., 2011). The mtDNA contains 15 protein-coding genes, large and small ribosomal subunits, 27 tRNAs and 8 unknown open-reading frames. Protein-coding genes residing on the mtDNA include *atp6*, *atp8*, *atp9*, *cox1-3*, *cytb*, *nad1-6*, *nad4L* and *RNA-Pol*. The presence and relative order of these genes is not highly conserved with closely related species of the Dothideomycetes (Aguileta et al., 2014; Hane et al., 2011b). However within the species *Z. tritici* across multiple isolates there was relatively little sequence variability (Zhan et al., 2003). Polymorphic sequences were identified between the mtDNA of the two sequenced isolates and used to generate markers for PCR screening across numerous isolates – an important genomic resource for studying the emergence and distribution of fungicide resistance. The mtDNA sequence data has also been a useful tool for tracking the history of the co-evolution of *Z. tritici* and its wheat host (Torriani et al., 2011; Zhan et al., 2004).

3. Gene function

Annotations of gene structure and function for *Z. tritici* IPO323 (Goodwin et al., 2011) are downloadable from JGI Mycocosm (Grigoriev et al., 2013) and EnsemblFungi (Kersey et al., 2014) (Table 1). As part of the initial genome survey study, potential carbohydrate-active enzymes (CAZymes) encoded by the gene content of *Z. tritici* were annotated, highlighting a distinctive reduction in cellulose and other cell-wall degrading enzymes relative to other plant pathogens (Goodwin et al., 2011). This observation supported a model of "stealth pathogenesis" for *Z. tritici*, in which CAZyme activity is reduced – with a compensatory shift toward protein degradation (Goodwin et al., 2011) – in conjunction with other adaptations for avoidance of triggering host-defenses (Lee et al., 2014). Morais do Amarai et al. subsequently used the gene annotations generated by Goodwin et al. to bioinformatically predict genes encoding secreted proteins and assign additional functional annotations. The predicted secretome (Morais do Amaral et al., 2012) comprises 492 proteins, with 321 possessing some level of functional annotation and 171 with no functional annotation.

4. Gene expression

An early transcriptome resource for *Z. tritici* was generated by Kema et al., comprising Expressed Sequence Tag (EST) libraries for IPO323 from 7 *in vitro* and 3 *in planta* growth conditions (Kema et al., 2008) (Table 1). Limitations around capture of fungal transcripts in mixed plant samples were overcome by purifying via hybridisation against IPO323 genomic DNA. A total of 27,007 ESTs were clustered into 9190 unigene loci, totaling 5.2 Mb. Though a comparable number of ESTs were typically sequenced in similar fungal genomics studies of the day, a very high representation of loci was achieved due to the high number of libraries from diverse growth conditions.

Recently, high-coverage whole-transcriptome expression data for the *Z. tritici*-wheat interaction has been facilitated by next-generation sequencing (RNA-Seq) (Table 1). Yang et al. have generated 18 Gbp of raw RNA-Seq data, comparing expression across the infection and necrotrophic growth stages of *Z. tritici* at 4, 10 and 13 days post-infection (dpi), as well as the corresponding host responses in *Triticum aestivum* [BioProject: 196595] (Yang et al., 2013). This study expanded upon the predicted secretome of Morais do Amarai et al., highlighting 313 genes of IPO323 that were highly expressed during early and late infection. A second RNA-Seq study by Kellner et al., generating 117.8 Gbp of RNA-Seq data, followed up with an in-depth comparison of *Z. tritici* expression during infection of two hosts: *T. aestivum* and *Brachypodium distachyon* [NCBI GEO: GSE54874] (Kellner et al., 2014). Differential expression patterns were observed across core and accessory chromosomes of *Z. tritici*. Kellner et al. observed no host-specific gene expression on accessory chromosomes during early infection and 25 wheat-specific genes during late infection (13 dpi). Notably, neither study had used the wealth of exon structure information afforded by RNA-Seq to re-annotate the initial set of gene predictions presented by Goodwin et al. (2011) (which relied heavily on *in silico* gene predictions supplemented with limited EST and homology supporting data). Consequently, despite considerable accumulation of knowledge of conditional gene-expression, a reasonable level of error in the prediction of gene loci, their exon structure and their translated sequences has persisted in the gene-based datasets of *Z. tritici* for a few years. However, RNA-seq data generated in a recent transcriptome study (Rudd et al., 2015) has been used in-house by the same research group to improve the accuracy of gene annotations in *Z. tritici* (annotations available from EnsemblFungi) (Kersey et al., 2014). The study itself by Rudd et al. has provided further insight into the wheat-*Z. tritici* interaction. RNA-Seq was used to profile expression at five infection time points (symptomless growth: 1 and 4 dpi; host-cell death: 9 and 14 dpi; and asexual sporulation: 21 dpi), detecting expression of at least 80% of predicted loci and identifying >3000 *Z. tritici* genes and >7000 wheat genes as differentially expressed during infection. Variation in the contribution of chromosomes to changes in expression was observed, with CCs exhibiting the highest overall and most significant changes in expression. Conversely ACs exhibited minimal gene expression and few differentially expressed genes. Genes encoding candidate effector proteins were found to be up-regulated *in planta* and expression data was used provide additional supporting evidence for the "top" candidates. Incorporating the above with profiles of differential metabolites over the same time course, Rudd et al. also observed that the switch from latent to necrotrophic growth and reproduction coincided with the activation of plant defense responses and a switch in *Z. tritici*'s nutrient source from lipids and fatty acid stores to carbohydrate metabolism.

5. Genome mutation, adaptation and pathogenicity

ACs have been described by Croll and McDonald as a "cradle for adaptive evolution". This refers to their potential for retention of higher levels of mutation over time, due to low impact on fitness and correspondingly lower selective pressures (Croll and McDonald, 2012). A two-speed rate of evolution across CCs and

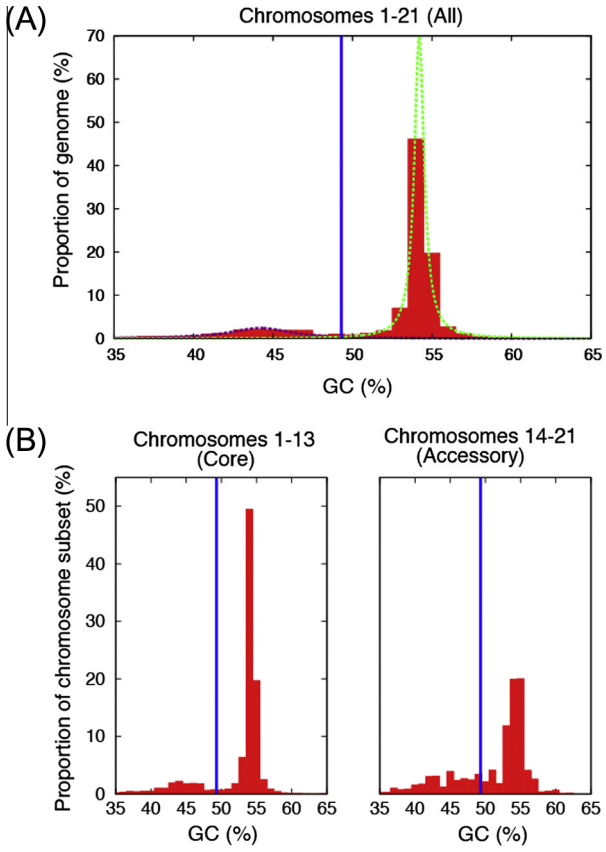

Fig. 1. Histograms of the GC content of segmented regions of the *Z. tritici* IPO323 genome assembly, and the relative proportion of the genome vs GC content. (A) The dotted lines show the two components of the Cauchy mixture model fit to the data. The solid vertical line shows the GC content selected to categorize segments, corresponding to the intersection between the two components of the mixture model. (B) Relative proportions of the *Z. tritici* IPO323 CC and AC chromosome subsets corresponding to ranges of GC content from 35% to 65% (red), illustrating depleted GC content within accessory chromosomes. The solid vertical line (blue) shows the GC content selected to categorize segmented regions of the genome as AT-rich or GC-equilibrated.

ACs allows pathogens like *Z. tritici* to retain its core gene content governing its viability and metabolism, whilst at the same time innovating and mutating genes rapidly in response to host-defences and control strategies. Plant pathogenicity genes have previously been observed to reside on the ACs of other plant-pathogenic fungi species, including *F. oxysporum* f. sp. *lycopersici* (Ma et al., 2010), *F. solani* (Coleman et al., 2009). Despite this, no *Z. tritici* pathogenicity genes have been mapped to ACs (Goodwin et al., 2011) with recent work reporting eight QTLs, all of which mapped to CCs (Mirzadi Gohari et al., 2015).

Z. tritici ACs are richer in repetitive DNA relative to CCs and also undergo recombination (including mesosyntenic rearrangements) more frequently (Croll et al., 2013). The higher repetitive DNA content of ACs, combined with active RIP, can also lead to the rapid mutation of non-repetitive genes flanking RIP-targeted repeats. In the related Dothideomycete plant pathogen *Leptosphaeria maculans* – which is well known for widespread distribution of AT-rich regions (syn. AT-rich isochores) within its genome – the "leakage" of RIP into neighboring non-repetitive has been demonstrated to accelerate the rate of non-synonymous mutation in avirulence genes arranged in a repeat-proximal configuration (Fudal et al., 2009; Hane et al., 2015; Van de Wouw et al., 2010). The *Z. tritici* genome has a similar abundance of AT-rich regions across its genome, particularly within its ACs, which may also have contributed to gene innovation and diversification over time. To assess

Table 1
Summary of sequence and bioinformatic resources available for *Z. tritici*.

Resource	Repository	Reference
EST libraries (IPO323)	EMBL (multiple accessions)	Kema et al. (2008)
Nuclear genome (IPO323)	NCBI Nucleotide: ACPE00000000.1	Goodwin et al. (2011)
Mitochondrial genome (IPO323 & STBB1)	NCBI Nucleotide: EU090238	Torriani et al. (2011, 2008)
Gene annotation (IPO323)	JGI (www.jgi.doe.gov)	Goodwin et al. (2011), Grigoriev et al. (2013)
	EnsemblFungi	Kersey et al. (2014)
Predicted secretome (IPO323)	n/a	Morais do Amaral et al. (2012)
Gene expression: RNA-Seq data for host and pathogen during wheat infection	NCBI BioProject: 196595	Yang et al. (2013)
	NCBI BioProject: 278138	Rudd et al. (2015)
Gene expression: RNA-Seq data for wheat infection vs *B. distachyon* infection	NCBI GEO: GSE54874	Kellner et al. (2014)
Mapped QTLs and effector candidate list (IPO323)	n/a	Mizardi Gohari et al. (2015)
Resequencing	n/a	Croll et al. (2013)_ McDonald et al. (1991)

the prevalence of AT-rich regions in *Z. tritici*, the genome was recursively segmented into regions of differing GC content using Jensen-Shannon divergence (Bernaola-Galván et al., 1996; Elhaik et al., 2010a, 2010b) (stopping criteria: minimum segment length of 1000 bp, t-test (5% significance level) on adjacent average GC values, similar to Oliver et al. (2004). Segmented genome regions were classified according to their constituent GC content. A mixture of two Cauchy distributions was fit to the data using expectation maximization, which identified two peaks centered at 54.2% and 44.2% GC. This allowed a GC boundary to be defined at 49.3% GC in order to distinguish "GC-equilibrated" from "AT-rich" DNA regions (Fig. 1A, Supplementary Data File 1). The AT-rich regions are substantially shorter than GC-equilibrated regions, with average lengths of 10.8 kbp and 48.5 kbp respectively. AT-rich regions were observed on all chromosomes and comprised 18.2% of the total genome length, however the proportion differed between ACs to CCs with AT-rich regions comprising 31.9% in ACs and 16.3% in CCs (Fig. 1B). The majority of annotated genes reside within GC-equilibrated regions, with an overall gene density of 334 genes/Mbp. In contrast, 91 genes have $\geqslant$50% of their length within AT-rich regions and are comparatively sparsely distributed at 12.6 genes/Mbp. Studies of related Dothideomycete plant pathogens, e.g. *Leptosphaeria maculans* and *Passalora fulva*, contain many secreted and/or effector-like genes associated with similar AT-rich regions (de Wit et al., 2012; Rouxel et al., 2011; Van de Wouw et al., 2010). As such, although genes within AT-rich regions contribute little to the overall gene content or gene-expression of *Z. tritici*, they may still play some role in pathogenicity and adaptability.

6. Conclusion

The sum of sequence and bioinformatic resources generated over the last decade for *Z. tritici* (Table 1) has significantly advanced knowledge of this fungal pathogen and its host-interactions. Foremost among these has been the generation of the whole-genome sequence – a solid foundation upon which multiple layers of additional information have been juxtaposed.

Various bioinformatic techniques have been applied to predicted gene regions and their protein products with a view to focussing on those with properties relevant to pathogenicity. This has been complemented by analysis of gene expression during infection, which has highlighted generic and host-specific pathogenicity genes in *Z. tritici*. The chromosome-length genome sequence has itself been a powerful tool in understanding genome mutation mechanisms influencing adaptation in *Z. tritici* and assessing the "genomic context" of its putative pathogenicity genes.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fgb.2015.04.011.

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